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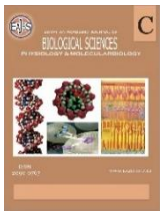

Effect of *Moringa Oleifera* Leaves Extract on Some Biochemical Responses of Diclofenac Treated Rats

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ABSTRACT

Background: Diclofenac sodium (DcNa) is one of non-steroidal anti-inflammatory drugs (NSAIDs) medicines which used to reduce pain and inflammatory condition. **Aim of the work:** The current study design to evaluate the possibility of *Moringa oleifera* (MO) leaves extract to improve the harmful effects of DcNa on different physiological parameters in albino rats. **Methods:** The study included 35 rats divided io to 5 groups: group I (control group), group II (DcNa group) animals treated with 10 mg of DcNa /kg b.w. daily, group III (MO groups) rats were treated with 350 mg of MO leaves extract /kg b.w., group IV (treated with DcNa then MO leaves extract) and group V (treated with MO leaves then DcNa). **Results:** The obtained results indicated that, treatment with diclofenac sodium showed significant decreases in antioxidant enzyme activities and significant increases in serum liver parameters, kidney function and lipid profile parameters. Moringa oleifera leaves extract administration to DcNa group caused an improvement in liver parameters and kidney functions, oxidative stress and lipid profile. **Conclusion:** Administration of diclofenac induced some adverse effects on some physiological parameters, which may be attributed to the oxidative stress induced by DcNa. MO leaves extract helps in ameliorate the negative effects caused by diclofenac sodium.

INTRODUCTION

The analgesic agents are known as non-steroidal anti-inflammatory drugs (NSAIDs) which are commercially available in sodium or potassium salt form (Ulubay *et al.,* 2018). These drugs (NSAIDs) are able to inhibit cyclooxygenase enzymes (COX-1 and COX-2) activities, which play a role in converting of arachidonic acids into prostaglandins; therefore, they block prostaglandin synthesis (Zerbini *et al*., 2011 and Atkinson and Fudin, 2020). Diclofenac sodium (DcNa) one of the NSAIDs. It is phenylacetic acid with strong analgesic, antipyretic, antibacterial and anti-inflammatory effects so it is extensively utilized for the treatment pain and inflammation linked to rheumatic diseases and non-rheumatic origin (Alabi and Akomolafe, 2020). DcNa at higher doses for a long period can cause severe toxicity in liver, kidney, bone marrow and cardiovascular in humans and animals (Owumi and Dim, 2019).

Natural plants are widely used globally and serve as a crucial component of healthcare. Among of these plants *Moringa* which traditionally used in herbal medicine (Gopalakrishnan *et al*., 2016).

Moringa (M) is a natural medicinal plant that belongs to family Moringaceae and it ranks among the most significant natural plants employed in herbal medicine.

Many parts in this plant used as food and herbal medicine because they are rich in bioactive compounds including nutrient and anti-nutrient substances. These nutrients including protein, essential amino acid, minerals, vitamins, fats, fibers and carbohydrates which help in combat malnutrition, supplement vitamin and minerals deficiencies (Aly *et al*., 2020 and Gharsallaha *et al*., 2023).

This paper highlights the potential *of Moringa oleifera* (MO) leaf extract in ameliorating the detrimental effects of diclofenac sodium (DcNa) on certain physiological indices in male albino rats.

MATERIALS AND METHODS

Experimental Animals:

Our study was conducted following approval from the Faculty of Science, Benha University (ZD/FSc/BU-IACUC/2022-17). Thirty-five male albino rats (*Rattus norvegicus*), weighing 130 ± 10 g, were obtained from the Helwan farm of the Egyptian Organization for Vaccines and Biological Preparations. The animals were randomly divided into five groups (seven rats per group). They were housed under controlled laboratory conditions (25 ± 2 °C, 12/12 h light-dark cycle) in clean cages, acclimatized for one week before the start of the experiment, and provided with a standard diet and water ad libitum. Aqueous Extraction of *Moringa oleifera* Leaves, *Moringa oleifera* (MO) leaves were purchased from Green Pharmacy, Egypt. The dried leaves were ground into a fine powder, and 100 g of the powder was extracted with 1000 mL of distilled water for 60 minutes. The extract was then filtered, and the aqueous phase was concentrated under reduced pressure using a rotary evaporator (Büchi R-110). The extract was stored at –10ºC until used.

Diclofenac Sodium:

Diclofenac sodium (DcNa) was obtained in tablet form from Novartis Pharma AG (Basel, Switzerland). It dissolved in distilled water for administration at an oral dose (10 mg/Kg) of DcNa for 7 days according to Owumi and Dim (2019).

Experimental Groups:

Group I: (control) Baseline group.

Group II: (DcNa group) 10 mg of DcNa /kg b.w. daily, was prepared in 0.5 mL distilled water and given once daily for 7 days.

Group III: (MO group) 350 mg of MO leaves extract /kg b.w. (Constance *et al*., 2020) was given daily for 21 days.

Group IV: Animals were administered DcNa, then subsequently treated with MO leaf extract.

Group V: Rats received MO leaf extract prior to DcNa administration.

All administrations were performed orally through gastric intubation.At the end of the experimental period, the rats were fasted for 12 hours prior to before anesthetized by diethyl ether inhalation and scarification. Blood was drawn and transferred into dry, sterile centrifuge tubes then centrifuged at 3000 rpm for 15 minutes using a Hittech centrifuge. The obtained sera were separated and stored at -20 °C for subsequent biochemical analysis. The liver was excised, rinsed with ice-cold phosphate-buffered saline (PBS, pH 7.2) to remove excess blood, and homogenized in ice-cold PBS. The homogenates were centrifuged at 5000 g for 5 minutes, and the resulting supernatants were stored at −20 °C until used for the determination of liver antioxidant markers and kidney injury molecule-1 (KIM-1).

Biochemical Evaluation:

The determination of antioxidant activity was performed spectro-photometrically with BioVision assay kits (BioVision Inc., California, the United States of America).Serum Lipid Profile was determined spectrophotometrically using SPECTRUM kit (Egyptian Company for Biotechnology "Spectrum Diagnostics", Cairo, Egypt)

Serum aspartate aminotransaminase (AST), alkaline phosphatase (ALP), alanine aminotransferase (ALT), and gamma-glutamyl transferase (GGT) were determined spectrophotometrically using commercial kits (Spectrum Diagnostics, Egyptian Company for Biotechnology, Cairo, Egypt).

The concentrations of serum total and

direct bilirubin were determined spectrophotometrically by using BiSTEP kit (#222 001, ARKAN ScienTech, Albohira, Egypt).Serum creatinine, urea and uric acid were determined spectrophotometrically using BioSystems kit (BioSystems S.A., Barcelona, Spain).Kidney injury molecule-1 (KIM-1) was assessed by enzyme-linked immunosorbent assay (ELISA) using a Rat KIM-1 kit. (#MBS355395, MyBioSource, California, the United States of America)

Statistical Analysis:

The results are reported as mean ± SD for 7 readings. All statistical analyses were carried out using SPSS software, Version 22 (IBM software, Inc. Chicago, USA, copyright SPSS INC., (2011). Statistical analysis was carried out using one-way analysis of variance (ANOVA). Differences among means were evaluated using Duncan's multiple range test, considering $P < 0.05$ as statistically significant.

RESULTS

Liver Antioxidants Enzymes:

Compared to all groups, DcNa group showed significant decreases in liver super oxide dismutase (SOD), catalase (CAT) and Glutathione (GSH) activities while a significant increase was observed in liver malondialdehyde (MDA) levels. Rats in Group III, treated with MO leaf extract, exhibited non-significant increases in hepatic SOD and GSH activities while liver MDA level decreased non-significantly compared to control group. Treatment with DcNa then MO leaves extract caused significant elevation in liver activities of SOD, CAT and GSH compared to DcNa treated group only while caused a significant reduction in liver MDA level compared to DcNa and MO leaves extract then DcNa treated groups (Table 1).

Table 1: Effect of *Moringa oleifera* "MO" leaves extract on liver antioxidants; superoxide dismutase (SOD), catalase (CAT), malondialdehyde (MDA) and reduced glutathione (GSH) of rats treated with diclofenac sodium.

Groups / Parameters	Group I	Group II	Group III	Group IV	Group V
SOD (U/mg)	6.37± 0.03^{a}	3.28± 0.40^{c}	6.51± 0.06^{a}	4.59± 0.15^{b}	4.91± 0.22^{b}
CAT (mU/mg)	5.15± 0.12^{b}	2.18± 0.16^{e}	5.63± 0.08^{a}	3.31± 0.19^{d}	3.75± 0.26^{c}
MDA (nmol/mg)	2.03± 0.06^{d}	4.84± 0.11^{a}	2.00± 0.06^{d}	4.19± 0.12^{c}	4.44± 0.20^{b}
GSH (µmol/mg)	60.09± 1.33^{a}	34.20± 0.81^{d}	61.39± 0.80^{a}	45.71± 1.13^{c}	48.31± 0.61^{b}

All data expressed as mean ±SD for 7 rats.
abcd = values with different letters are significantly different ($P < 0.05$).

Lipid profile:

DcNa treatment caused significant increases in serum triglyceride (TG), total cholesterol (TC) and Low-Density Lipoprotein-cholesterol (LDL-C) levels compared to control group and other treated groups while showed a significant decrease in serum high Density Lipoprotein-cholesterol (HDL-C) level compared to control group, MO leaves extract treated group. As compared to DcNa treated group, rats treated with MO leaves extract showed significant reduction in serum TG, TC and LDL-C levels while showed a significant elevation in HDL-C level (Table 2).

Table 2: Effect of *Moringa oleifera* "MO" leaves extract on lipid profile parameters of rats treated with diclofenac sodium.

Groups / Parameters	Group I	Group II	Group III	Group IV	Group V
Triglyceride (mg/dl)	155.40±3.91^{cd}	274.70±2.77^{a}	145.40±10.69^{c}	165.80±5.71^{c}	228.60±16.33^{b}
Total Cholesterol (mg/dl)	61.20±1.92^{c}	98.80±9.78^{a}	61.80±3.96^{c}	70.00±3.16^{b}	76.80±8.10^{b}
LDL-C (mg/dl)	7.76±0.49^{b}	14.03±0.72^{a}	7.55±0.49^{b}	5.55±0.93^{c}	2.78±0.80^{d}
HDL-C (mg/dl)	34.52±1.39^{a}	23.24±3.54^{c}	33.06±1.01^{a}	22.62±2.18^{c}	28.32±1.18^{b}

All data expressed as mean ±SD for 7 rats.
abcd = values with different letters are significantly different ($P < 0.05$),

Liver Function Parameters:

Serum AST and ALT levels were significantly elevated in rats treated with DcNa compared with the control and all other treated groups (Table 3). Treatment with MO leaves extract caused a significant reduction in serum AST level compared to control group and other treated groups. Rats in Group IV exhibited a significant decrease in serum AST levels compared with the DcNa-treated group, while showing a significant increase relative to the MO leaf extract–treated group. Rats received MO leaf extract prior to DcNa administration in group V showed significantly reduction and elevation in serum AST level compared to group II and group III respectively.

Table 3: Effect of *Moringa oleifera* "MO" leaves extract on liver function parameters; serum aspartate aminotransaminase (AST), alkaline phosphatase (ALP), alanine aminotransaminase (ALT), gamma-glutamyl transferase (GGT), total bilirubin and direct bilirubin of rats treated with diclofenac sodium.

Groups / Parameter	**Group I**	**Group II**	**Group III**	**Group IV**	**Group V**
AST (U/L)	149.62±6.41^{b}	174.40±17.28^{a}	133.04±5.41^{c}	151.20±9.88^{b}	154.05±14.61^{b}
ALP (U/L)	191.50±12.27^{b}	282.04±7.69^{a}	191.51±15.27^{b}	155.60±7.92^{c}	146.40±17.60^{c}
ALT (U/L)	32.37±4.92^{b}	53.60±2.30^{a}	35.32±1.01^{b}	35.20±2.58^{b}	32.27±1.23^{b}
GGT (U/L)	16.10±0.65^{a}	16.74±1.11^{a}	12.85±1.79^{b}	15.56±1.67^{a}	15.78±1.04^{a}
Total Bilirubin (mg/dl)	0.65± 0.05^{c}	0.85±0.05^{a}	0.13±0.03^{d}	0.75±0.04^{b}	0.73±0.02^{b}
Direct Bilirubin (mg/dl)	0.10±0.01^{c}	0.21±0.01^{a}	0.11±0.01^{c}	0.16±0.01^{b}	0.15±0.01^{b}

All data expressed as mean ±SD for 7 rats.
abcd = values with different letters are significantly different ($P < 0.05$).

Administration of diclofenac resulted in a significant elevation of serum ALP levels relative to all other experimental groups. Treatment with MO leaves extract produced a non-significant alteration in serum ALP levels compared to the control group. Serum GGT level showed non-significant increase in rats treated with DcNa compared to control group and other treated groups except MO leaves extract treated group in which GGT level decreased significantly relative to all groups.

Treatment with DcNa in group II resulted in significant elevation in serum total

bilirubin and direct bilirubin levels compared to all other experimental groups, while treatment with MO leaves extract exhibited a considerable reduction in serum total bilirubin level compared to all groups.

Kidney Function Parameters:

Data in Table (4) revealed that DcNa treatment caused significant increases in serum creatinine, uric acid and urea levels relative to all groups. Administration of MO leaves extract to group III resulted in a significant lowering of serum creatinine levels compared to all experimental groups while serum uric acid and urea decreased significantly compared to all groups except MO leaves extract then DcNa-treated group in which the decrease was non-significant.

Kidney Injury Molecule-1 (KIM-1):

DcNa treatment resulted in a substantial elevation in KIM-1 levels relative to the control and all other treated groups. In relation to the control group, level of KIM-1 in MO leaves extract-treated group was increased non-significantly. Rats in groups IV and V showed a substantial lowering in KIM-1 level in comparison with the DcNa-treated group only (Table 4).

Table 4: Effect of *Moringa oleifera* "MO" leaves extract on kidney function; serum creatinine, uric acid and urea of rats treated with diclofenac sodium.

Groups / Parameter	Group I	Group II	Group III	Group IV	Group V
Creatinine (mg/dl)	0.60±0.02^{c}	0.71±0.01^{a}	0.56±0.01^{d}	0.64±0.01^{b}	0.63±0.01^{bc}
Uric acid (mg/dl)	1.91±0.17^{b}	3.31±0.13^{a}	1.39± 0.02^{d}	1.72±0.13^{bc}	1.60±0.13^{cd}
Urea (mg/dl)	54.73±3.38^{b}	120.13±5.14^{a}	47.80±1.15^{c}	57.25±3.53^{b}	44.70±2.75^{c}
KIM-1 (pg/mg)	40.50± 1.22^{c}	93.99± 1.78^{a}	42.35± 0.69^{c}	74.92± 1.62^{b}	73.02± 1.73^{b}

All data expressed as mean ±SD for 7 rats.
abcd = values with different letters are significantly different ($P < 0.05$).

DISCUSSION

Diclofenac, a phenylacetic acid derivative, is a non-steroidal anti-inflammatory drug (NSAID) that belongs to the acetic acid class. Diclofenac is used in the therapy of acute pain, chronic pain and inflammation (Boeing *et al*, 2024).

Many cellular injuries and adverse effects on many different body tissues can be caused by administration of diclofenac, it can induce alterations in renal function, elevate blood pressure, cause hepatic injury, inhibit platelet function, and contribute to gastrointestinal and cardiovascular disorders. Many cellular structural macromolecules (protein, lipid, carbohydrate and DNA) can be affected by diclofenac treatment which can perturb the oxidant/antioxidant balance in cells and leading to a significant rise in reactive oxygen species (ROS) and a concomitant decrease in antioxidant levels (Mraisel *et al*, 2024).

ROS generation, oxidative stress, oxidative of protein, peroxidation of lipid, mitochondrial dysfunction and reduction of endogenous antioxidant capacity are the main mechanisms of DcNa-induced hepato-nephrotoxicity and apoptosis (El-Baz *et al*., 2022). The current findings indicate that the administration of DcNa induced a significant decline in hepatic antioxidants (SOD, CAT and GSH) while inducing a significant increase in hepatic MDA level in comparison to all other experimental groups. Our results corroborate with El-Shopakey and El-Azab (2021) who reported that DcNa caused remarkable diminutions in hepatic SOD, CAT and GSH activities while caused a marked increase in MDA level in liver of DcNa-treated rats.

Superoxide dismutase (SOD), the principal antioxidant enzyme within cells, is

considered as the first line of defense against oxidative stress by stimulating the dismutation of two molecules of superoxide anion to molecular oxygen and hydrogen peroxide (H_2O_2) that might eventually converted into water and oxygen by the action of CAT (Ighodaro and Akinloye, 2018). SOD and CAT help to protect the tissues from inflammation and lipid peroxidation thereby preventing cellular damages which caused by free radicals. Therefore, a reduction in activities of these enzymes may lead to various deleterious effects because accumulation of high toxic metabolites, superoxide and hydrogen peroxide in some organs in rats treated with DcNa (Owumi and Dim, 2019).

Glutathione (GSH), the predominant intracellular thiol, functions as a key non-enzymatic antioxidant, providing essential protection against oxidative stress and chemically induced hepatic damage (Basu *et al*., 2012). The GSH acts as scavenging free radical species such as H_2O_2 and hydroperoxides (ROOH), reduces xenobiotic toxicity and maintains membrane protein thiols (El-Shafey *et al*., 2018). A reduction in hepatic GSH activity after treatment with DcNa may be due to the induced toxicity in liver and the conjugation of diclofenac with GSH to form diclofenac-SG adduct and consumption of GSH by the glutathione-dependent enzymes or the excessive utilization of GSH by cells to counteract the increased generation of reactive oxygen species "ROS" (Abiola *et al*., 2019). This may confirm the impairment of antioxidant system and therefore increased the exposure of tissues to oxidative stress (Alabi and Akomolafe, 2020).

Malondialdehyde (MDA) level is a widely recognized biomarker of lipid peroxidation and protein oxidation confirming the oxidative stress (Heidarian *et al*., 2017). Diclofenac one of the most potent NSAIDs in inhibiting mitochondrial electron transport chain complex-I which leads to leakage of electron from the respiratory chain and impaired mitochondrial function (Sandoval-Acuña *et al*., 2012). The leaked electron causes partial reduction of molecular oxygen leading to formation of ROS which is directly transformed by the mitochondrial superoxide dismutase to H_2O_2 which escapes through permeable membrane of mitochondria causing damage in cellular macromolecules (DNA, lipids, protein, and carbohydrates) and therefore increases MDA level.

MO scavenges the free radical and causes elevation in activities of antioxidant defense system and reduces level of malondialdehyde and therefore protects cells from ROS and oxidative damage (Karthivashan *et al*., 2016). The data obtained in this study revealed that treatment with MO leaves extract after and before DcNa caused marked elevations in hepatic and renal antioxidants (SOD, CAT and GSH) activities while exhibited marked decline in MDA level in liver and kidney of rats relative to DcNa-treated group only. This may be attributed to occurrence of high content of phenolic compounds in MO leaves (Dhakad *et al*., 2019). Specific phenolic compounds can upregulate phase II drug metabolism enzymes, enhancing the elimination of oxidizing species and suppressing cytochrome P450 activities. Flavonoids and phenolic acids, the main phenolic compounds, are hydrogen molecules donors that have scavenging effect on the free radicals which help to protect tissues from damage of ROS (Alqahtani and Albasher, 2021). This effect also, result from the abundant presence of vitamin A, C and E in leaves of MO where vitamin A reduces ROS and regenerates membrane-bound antioxidants and vitamin C functioning as a free radical scavenger and helps in inhibition of lipid peroxidation in liver tissue and also vitamin E protects membrane lipoprotein from oxidative damage (Vergara-Jimenez *et al*., 2017 and El-Sayed *et al*., 2021).

Analysis of the results indicates that DcNa caused significant increases in serum TG, TC and LDL-C levels while caused a significant decrease in serum HDL-C level. These results may be attributed to harmful effects of DcNa which lead to hepatobiliary

abnormalities and altered cholesterol metabolism. Our results are consistent with those of Hassan *et al.* (2017) who demonstrated that the treatment with DcNa caused significant increases in the levels of TG, TC and LDL-C levels while caused a significant decrease in serum HDL-C level that is an indication of dyslipidemia. TG mainly represent the most lipids which accumulate in the hepatic cells but are transported by lipoproteins to peripheral tissues. However, hepatotoxins which cause deficiency of lipoproteins, prevent the transporting of TG, therefore they remain in the liver cells and cause lysis of the cells that lead to an increase in serum level of TG (Orinya *et al*., 2016). Oxidative stress and toxic effect which caused by an induction of DcNa caused disorders in fat metabolism. These disorders caused disturbances in digestion and absorption process as a consequence of inhibited steroid and bile salt secretion/excretion and this was indicated by high level of TC (Jasim *et al*., 2022). High level of LDL-C refers to increased cholesterol level in blood stream, and this increases the risk of heart disease (Onwe *et al*., 2015).

Amelioration and protection groups in the current study showed significant decreases in serum TG, TC and LDL-C levels. HDL-C level in serum decreased non-significantly and increased significantly in amelioration and protection groups, respectively when compared with DcNa-treated group. This may be attributed to that MO contains sterols which help in reduction of cholesterol level in blood by LDL-C lowering effect, inhibiting the reabsorption cholesterol, and, enhancing its elimination through feces as neutral steroids (Ghasi *et al*., 2000). These observations corroborate with Zeidan *et al*. (2019) who described that administration of MO leaves extract to diabetic male rats caused marked reduction in serum TG, TC and LDL-C levels while causing a substantial increase in HDL-C level that may be due to the natural antioxidant content, such as polyphenol present in MO which could ameliorate dyslipidemia. This may be attributed to that MO leaves contain β-sitosterol that decreases absorption of intestine for dietary cholesterol (Mbikay, 2012). Also, leaves of MO are rich with alkaloids which might mediate the action of hypolipidemic either by up regulation of activities of lipolytic enzymes or by stimulating the excretion of faecal bile acid. The decrease in serum TC, TG and LDL-C levels may be owing to the suppression of cholesterol synthesis causing a decline in liver intracellular sterols (Dhakad *et al*., 2019 and Ghasi *et al*., 2000).

Treatment with DcNa causes hepatocellular necrosis the production of substantial amounts of oxidative free radicals, such as superoxide anions and the highly reactive hydroxyl radical. These radicals trigger lipid peroxidation of cellular membranes, oxidizing unsaturated fatty acids, which in turn reduces membrane fluidity and disrupts membrane structure and function (Simon and Evan Prince, 2018). The damage in hepatic cell membrane caused leakage of liver enzymes which are normally located in the cytosol into circulation (Arote *et al*., 2014). These enzymes represent the rationale for evaluating liver dysfunction and diseases (Buechter and Gerken, 2022). El-Shopakey and El-Azab (2021) and Hassan *et al.* (2021) mentioned that inhibition of the antioxidant defense system and elevation of oxidative stress and mitochondrial damage are the principal elements participating in DcNa toxicity.

In the current study, DcNa administration caused marked elevation in liver enzymes (AST, ALP, ALT, total bilirubin and direct bilirubin) as compared to the control, other experimental group and caused a non-significant elevation in serum GGT level relative to all groups except group III. These results were in agreement with El-Hadary and Ramadan (2019) who reported that administration of DcNa caused significant increases in serum level of AST, ALT, ALP, total bilirubin and direct bilirubin. The increment in ALP and bilirubin levels after administration of diclofenac caused damage to biliary duct in liver (Brandoni *et al*., 2012).

In the present study, amelioration and protection groups showed significant decreases in serum AST, ALP, ALT, direct bilirubin and total bilirubin levels compared to DcNa-treated group while serum GGT level decreased non-significantly relative to control group and DcNa-treated group. The decrease in liver enzymes may be attributed to that MO extract has a vital role to protect hepatocellular membrane, inhibit liver cell damage and reduce liver enzymes leakage into circulation (Aly *et al*., 2020). The author reported that significant drop in liver enzymes may be due to MO leaves extract has hepatoprotective property due to the high content of antioxidants such as flavonoids and phenolic acid which help into inhibition free radical activity. The significant reduction in serum bilirubin level after administration of MO leaves extract suggests the effectiveness of the extract to relieve or protect membrane of RBCs from damage (Suleiman *et al*., 2017).

Diclofenac has an adverse effect on renal physiology where it inhibits the production of renal prostaglandin, decreases vasodilation and increases resistance in renal afferent, therefore causes decrease in the glomerular capillary pressure and the glomerular filtration rate relative to normal values. This exhibits as acute renal dysfunction, electrolyte and fluid disorders and renal papillary necrosis and interstitial nephritis (Bancroft and Gamble, 2008 and Abiola *et al*., 2019). Serum levels of creatinine, uric acid and urea are the most important biomarkers used for evaluation the disturbance in renal function, therefore the increase in these parameters may show renal impairments (Moradi *et al*., 2020). Our results showed significant increases in serum levels of uric acid, creatinine and urea in rats treated with DcNa which indicated the toxic and harmful effect and oxidative stress of DcNa on renal physiology. These results were in agreement with El-Hadary and Ramadan (2019) who reported that serum urea, uric acid and creatinine levels increased significantly in rats treated with DcNa that may be because of maintenance of glomerular activity together with enhanced oxidative stress. Primarily creatinine and urea (metabolic waste products) excreted by the kidney in the urine, but DcNa treatment induced reduction in elimination of creatinine and urea, thus leads to elevation in serum creatinine and urea levels. The rate of creatinine and urea excretion is related to glomerular filtration rate and any disturbance in nephron which effect on glomerular filtration rate (Owumi and Dim, 2019). The results in current study also agreed with Al-Kuraishy *et al*. (2019) and Alabi and Akomolafe (2020) who concluded that administration of diclofenac marked elevation in creatinine and urea levels. This may be due to the reduce in elimination of metabolized products causing severe disruption of renal tubular function (Mostafa *et al*., 2020). High serum level of uric acid after administration of DcNa may be due to uric acid (electron donor) donates electrons in defense against oxidative damage caused by free radical (Abiola *et al*., 2019).

Data in present study indicated marked decreases in all renal biomarkers (uric acid, creatinine and urea) in rats treated by MO leaves extract. Amelioration and protection groups in the current study showed substantial reductions in levels of creatinine, uric acid and urea. *Moringa* has nephroprotective effects because it contains a good source for antioxidants including phytochemical constituents. Antioxidant compounds of plant can perform their safety roles in living organisms by stopping increment or counterbalancing the free radicals (Verma *et al*., 2009). MO have polyphenols and tannins which have anti-inflammatory and antioxidant effect causes decrease in serum creatinine and urea levels and enhanced kidney function (El-Shafey *et al*., 2018). MO leaves extract has protective effect where it prevents kidney damage which indicated by a significant decrease in all kidney function parameters (El-Hadary and Ramadan, 2019). These results also agreed with El-Hamalawy *et al*. (2022) who reported that addition of (TMX) with MO leaves extract induced significant decreases in serum levels of urea, uric acid and creatinine that

may be attributed to that MO leaves are rich with essential fatty acids that show its antioxidant properties and protect tubular cell membrane form oxidative stress.

The kidney injury molecule-1 (KIM-1) is the principal biomarker that reflects tubular dysfunction of the kidney. These results revealed that DcNa caused marked elevation in tissue's level of KIM-1 when compared to all groups. This may be attributed to oxidative stress which caused by DcNa induction. These results are in the same line with Hussien *et al*. (2019) and Abdou *et al*. (2021) who reported that serum KIM-1 level increased significantly after administration of DcNa. Since DcNa induces acute kidney injury (AKI), the increase in serum level of KIM-1 is considered a biomarker for the diagnosis of this disease. AKI is a renal-specific condition characterized by structural and functional renal damages, where it leads to disturbance in renal functions (Hussien *et al*., 2019 and Zdziechowska *et al*., 2020). Nephrotoxic agents promote renal inflammation that causes glomerulonephritis and interstitial nephritis. In addition, the results were in same line with and Alorabi *et al*. (2022) who concluded that animals treated with DcNa showed a considerable elevation in serum KIM-1 level.

Amelioration and protection groups showed a significant reduction in tissue KIM-1 level relative to DcNa-treated group only. These results may be associated with MO leaves extract have higher content of antioxidants which reduce ROS, thereby improving kidney function markers, including KIM-1. These results agreed with Ismaiel *et al*. (2019) who concluded that rats treated with MO extract then treated with gentamicin-induced nephrotoxicity, showed a significant reduction in KIM-1 level. The therapeutic effect of MO leaves extract help in protection the kidney and prevents renal damage (Abdou *et al*., 2019). Also, these results support the findings of Adedapo *et al*. (2020) who indicated that treatment with MO extract then injected with glycerol which induced acute kidney damage and reduction in expression of renal KIM-1 level. In addition, the present results lie in the same line with Abd-Elhakim *et al*. (2021) who confirmed that rats treated with MO extract then treated with melamine-induced hepatorenal damage and rats co-treated with extract of MO and melamine showed a significant reduction in KIM-1 level in renal tissue relative to melamine-treated rats, therefore MO has protective effect against toxic damage induced by melamine.

In conclusion, the current research indicated that MO leaves extract exhibits ameliorative and protective effects against the harmful impacts of DcNa, which may be attributed to its high antioxidant content. It was concluded that the protective role of MO leaves extract was more effective than the ameliorative role of them.

In the recommendation, extract of MO leaves may be useful in improving the hepato-renal toxic effects induced by DcNa, especially when used MO leaves extract before DcNa.

Declarations:

Ethical Approval and Consent to Participate: The experimental protocol of this study was approved by the Institutional Animal Care and Use Committee (IACUC) of the Zoology Department, Faculty of Science, Benha University (Approval No. ZD/ FSc/ BU-IACUC/2022-17). All procedures were conducted in accordance with the National Research Council's Guide for the Care and Use of Laboratory Animals.

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Authors' Contributions: All authors' contributions to research design. Marwa, A.E. Abd-Elmaksoud, and Maha, G. Mohamed experimented, wrote and reviewed the paper. Mohamed N.E. Seddeik and Eman M.S. Shahen were responsible for analyzing and interpreting the data. The final manuscript was read and approved by all authors.

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